DATA EVALUATION RECORD
FISH LIFE-CYCLE TOXICITY TEST
§72-5

1. **CHEMICAL:** Novaluron — PC Code No.: 124002

2. **TEST MATERIAL:** Novaluron Technical — Purity: 99.9%

3. **CITATION:**

Author: Caunter, J.E., and T.D. Williams

Title: Novaluron technical: Determination of effects on the life cycle of the fathead minnow (*Pimephales promelas*)

Study Completion Date: March 11, 2002

Laboratories: Brixham Environmental Laboratory
AstraZeneca UK Limited
Brixham Devon, UK

Sponsor: Makhteshim Chemical Works Ltd.
P.O. Box 60
Beer Sheeva, Israel

Laboratory Report ID: AJ0047A

MRID No.: 45785805

DP Barcode: D287624

4. **REVIEWED BY:** Rebecca Bryan, Staff Scientist, Dynamac Corporation

Signature: Rebecca Bryan **Date:** 4/1/03

APPROVED BY: Christie E. Padova, Staff Scientist, Dynamac Corporation

Signature: C.E. Padova **Date:** 4/1/03

5. **APPROVED BY:** Bill Evans, Biologist, OPP/EFED/ERB - I

Signature: William Evans **Date:** 11/17/03

2013351

6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Pimephales promelas*

Age of Test Organism: <24 hours old

Definitive Test Duration: 315 Days

Study Method: Flow-through

Type of Concentrations: Mean-measured

7. CONCLUSIONS:

The 10-month chronic toxicity of Novaluron technical to the full life stage of Fathead Minnow (*Pimephales promelas*) was studied under flow-through conditions. Fertilized eggs (200 embryos/treatment, <24 hours old) of fathead minnow were exposed to the test material at nominal concentrations of 0 (negative and solvent controls), 0.09, 0.22, 0.52, 1.25, and 3.00 μg/L. The solvent was dimethylformamide (DMF) at a concentration of 2.86 μL/L. Mean-measured concentrations were <0.020 (LOD; controls), 0.061, 0.20, 0.44, 1.1, and 2.4 μg/L. The test system was maintained at 23.6 to 25.8°C and pH 7.10 to 7.89.

Following hatching on Day 4, alevins were reduced to 100 per treatment level. On Day 60 (approximately 8 weeks post-hatch), the juveniles were again reduced to 50 per treatment level. On Day 145, 16 adult fish per treatment level were paired off into breeding compartments. Starting on Day 159, hatchability trials and early life stage studies were performed for the F_1 generation. Both early life stage tests were conducted for 56 days post-hatch. The test was terminated after 315 days.

Endpoints assessed for the F_0 generation included hatching success, survival at 8 and 21 Weeks post-hatch, total length and wet weight at 21 and 44 Weeks post-hatch (gender-specific), total number of eggs produced, and the total number of eggs produced per female reproductive day. Endpoints assessed for the F_1 generation included hatching success, survival at 8 Weeks post-hatch, and total length and wet weight at 8 Weeks post-hatch.

No treatment-related effects were observed on any endpoint.

Since adequate raw data pertaining to survival of both generations and growth of the F_0 generation were not provided to verify the results of the study, this study does not fulfill guideline requirements of a fish life-cycle toxicity test [§72-5] and is classified **INVALID**. Furthermore, significant endpoints that were not assessed included: the time to hatch for both F_0 and F_1 generations, lengths of F_0 fish at 4 and 8 Weeks post-hatch, and survival of

F_0 fish at 4 Weeks post-hatch. This study may be upgraded to Supplemental status if appropriate raw data are provided; however, significant endpoints that were not assessed during the study prevents this study from fulfilling guideline requirements.

Results Synopsis:

NOEC: Not determined (Invalid study)
LOEC: Not determined (Invalid study)
MATC: Not determined (Invalid study)

Most Sensitive Endpoint: Not determined (Invalid study)

8. ADEQUACY OF THE STUDY:

A. Classification: Invalid

B. Rationale: Adequate raw data were not provided to verify the results of the study.

C. Repairability: This study may be upgraded to Supplemental status if appropriate raw data are provided; however, significant endpoints that were not assessed during the study prevents this study from fulfilling guideline requirements.

9. GUIDELINE DEVIATIONS:

1. De-chlorinated tap water was used as the dilution water.

2. The pH range (7.10 to 7.89) very slightly exceeded the recommended range (7.2-7.6).

3. The dissolved oxygen level range of 56 to 102% was slightly below the required range for aquatic studies of 60%.

4. The photo-period was based on dawn and dusk times in Evansville, Indiana. The photo-periods varied from 10 hours and 45 minutes/day to 15 hours and 45 minutes/day during the study instead of the recommended 16 hours light/8 hours dark photo-period.

5. Although observed, the time to hatch endpoint was not statistically assessed.

6. Attempts to measure lengths of F_0 fish at 4 and 8 weeks post-hatch were

unsuccessful, and therefore, these endpoints were not provided.

7. Replicate data were not provided for survival (F_0 and F_1 generations) or growth at Weeks 21 and 44 (F_0 generation). Therefore, statistical verification for these endpoints was not conducted.

10. SUBMISSION PURPOSE: This study was submitted to provide data on the toxicity of Novaluron to the life cycle of fathead minnows for the purposes of chemical registration.

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species Prefer Sheepshead minnow (*Cyprinodon variegatus*) or Fathead minnow (*Pimephales promelas*).	Fathead minnow (*Pimephales promelas*)
Source and Acclimation	Embryos were obtained from two brood stocks maintained for an unspecified period of time at Brixham Environmental Laboratory. The acclimation dilution water was only filtered through a 10 μm filter prior to use. There were no mortalities within 7 days prior to testing.
Age at beginning of test Embryos, 2 to 24 hours old	Embryos, <24 hours old

Guideline Criteria	Reported Information
Feeding Fish should be fed at least twice daily and should not be fed for at least 24 hours prior to test termination.	The larvae, juveniles, and adults were fed three times per day on weekdays and twice per day at weekends. The larvae were fed 3 mL of freshwater rotifers (*Brachionus plicatilis*) per replicate tank from hatch day to post-hatch day 7. On post-hatch day 7, the larvae were also fed 0.67 mL of live brine shrimp (*Artemia salina*) per fry. On post-hatch days 8 to15, the larvae were fed from 0.67 to 2.0 mL of live brine shrimp (*Artemia salina*) per fry. From post-hatch days 16 to 55, the larvae were fed with high protein pelleted fish food, *ad libitum,* once daily and 3.0 mL live brine shrimp. From post hatch day 56, the adults were fed frozen adult brine shrimp supplemented with high protein pelleted fish food. Fish were not fed at least 24 hours prior to sampling.
Embryo Exposure (4 to 5 Days) Embryos (≤24 hours old) from at least 3 separate spawns should be randomly distributed to embryo cups. A minimum of 50 embryos (≤24 hrs old) per replicate cup, 4 cups per treatment should be used. Parameters measured: · Survival of embryos · Time required to hatch · Hatching success · Survival of fry for 4 weeks Dead and fungused embryos should be counted and removed daily.	Days 0-4 Embryos (< 24 hours old) from two spawns were randomly distributed to embryo cups. Each cup contained 25 embryos, with two cups per replicate and four replicates per treatment level (total of 200 embryos per treatment). Parameters measured: · Survival of embryos · Hatching success Mortality and clinical signs of toxicity were made daily. Dead embryos were removed.

Guideline Criteria	Reported Information
Larval-Juvenile Exposure (From Hatch to 8 Weeks) After hatching, each group of larvae is randomly reduced to a minimum of 25 fish and released in replicate larval growth chambers. The random selection must include any fish that are lethargic or deformed. Parameters measured: · Fish survival (determined by counting the number of live fish in each replicate growth chamber weekly). · Total lengths (mm) of all fish at 4 and 8 weeks after hatching.	4-60 (hatch to approximately 8 weeks) When ≥90% of the embryos had hatched or 24 hours after first hatch, larvae were impartially thinned to 25 per replicate, with four replicates per treatment (100 embryos per treatment), and the larvae were transferred from the incubation cups to the progeny tanks. Parameters measured: · Survival of fry at 8 Weeks post-hatch

Guideline Criteria	Reported Information
Juvenile-Adult Exposure (From 8 weeks posthatch to the end of the spawning phase [32-40 weeks]) At 20-24 weeks after hatching, mature fish are placed in a spawning tank of the same concentration (4 males and 4 females randomly chosen and assigned). The spawning tank is divided into 4 individual spawning chambers with appropriate spawning substrates. The substrates are examined daily and embryos removed, counted, and recorded separately for each pair. For fathead minnow, adult exposure should be terminated when no spawning occurs for one week. For sheepshead minnow, testing should be terminated after spawning is observed for 2 weeks.	Days 60 to 315 (approximately 8 to 42 weeks post-hatch) On Day 60, juvenile fish were reduced to 25 per adult tank (50 total fish per treatment). On Day 145 (approximately 20 weeks post-hatch), one male and one female were impartially assigned to each spawning compartment with one spawning tile. There were four spawning compartments per replicate. Excess fish were divided into duplicate progeny tanks. The spawning substrates are examined daily and embryos were removed and counted. Adult exposure was terminated on Day 315. Parameters measured: · Survival of adult fish at 21 Weeks post-hatch · Number of eggs produced · Number of eggs/female/reproductive day · Total lengths (mm) and weight (g) at 21 and 44 Weeks post-hatch
Second Generation Embryo Exposure (4 to 5 days) 50 embryos from each conc. level are randomly selected and transferred to incubation cups for hatch. Use the same test procedures as those for parental generation. Embryos not selected are discarded.	Beginning on Day 159 (Hatchability tests) Spawnings of at least 50 embryos, from single females, were randomly selected and transferred to incubation cups for hatch. The same test procedures as those employed for the parental generation were used, and the same endpoints were measured. Two hatchability tests were performed per F_0 breeding pair.

Guideline Criteria	Reported Information
Second Generation Larval-Juvenile Exposure (From Hatch to 4-8 weeks) After hatching, 25 larvae are released in each growth chambers (2 chambers per treatment). Each group of 2nd generation fish is terminated 8 weeks after hatching. Fish are blotted, weighed, and measured before being discarded.	Days 159-291 (Early Life Stage tests) After hatching, 50 randomly selected larvae were released into each of two replicate test chambers (100 total larvae per treatment). Two early life stage tests were performed per adult (F_0) tank. Each group of F_1-generation fish was terminated 8 weeks after hatching. Fish were weighed and measured for total length.

Comments: None.

B. Test System

Guideline Criteria	Reported Information
Test Water Sheepshead Minnow 1. Natural seawater (sterilized and filtered) or a commercial mixture. 2. Natural seawater with a salinity of ≥15 parts per thousand (weekly range of salinity <6% and monthly pH range <0.8 pH units). Fathead Minnow 1. Reconstituted water or water from unpolluted well or spring (sterilized and tested for pollutants). 2. Hardness of 40 to 48 mg/L as $CaCO_3$ and pH of 7.2 to 7.6.	N/A 1. De-chlorinated tap water. The water was filtered with activated carbon and mesh filters, and de-chlorinated with sodium thiosulphate, and salts were added. The results of periodic analysis for selected contaminants were provided. 2. Hardness of 41.0 to 51.0 mg/L as $CaCO_3$ and pH of 7.10 to 7.89.
Test Temperature Sheepshead: 30°C. Fathead: 25°C and should not remain outside the range of 24 to 26°C for more than 48 hours.	N/A Range of 23.6 to 25.8°C.
Photoperiod 16-hour light/8-hour dark. Light intensity of 10-100 lumens at water surface.	Photoperiod based on dawn and dusk times in Evansville, Indiana. The photoperiods varied from 10 hours and 45 minutes of light/day to 15 hours and 45 minutes of light/day during the study (Appendix 2, p. 58). The light intensity ranged from 420 to 600 Lux.

Guideline Criteria	Reported Information
Dosing Apparatus 1. Intermittent flow proportional diluters or continuous flow serial diluters. 2. A minimum of 5 toxicant concentrations with a dilution factor ≤0.5. 3. One control should be used.	1. Continuous-flow diluter. 2. Five toxicant concentrations with a dilution factor of 0.4. 3. Negative and solvent controls were used.
Toxicant Mixing 1. Mixing chamber recommended but not required. 2. Test solution completely mixed before introduction into the test system (aeration should not be used for mixing). 3. Flow splitting accuracy must be within 10% and periodically checked.	1. A mixing chamber was used for each toxicant level. 2. Yes 3. Mixing and flow splitting chambers were checked twice per week.
Exposure System/Test Vessels Exposure tanks should be all glass or glass with a plastic or stainless steel frame (30.5 x 30.5 x 91.4 cm or 30.5 x 30.5 x 61 cm for fathead, and 45 x 90 x 26 cm for sheepshead). Larval chambers should have glass bottoms and drains that allow water to be drawn down to 3 cm. Test water depth in adult tanks and larval chambers should be a minimum of 15 cm.	Adult exposure tanks were 54 L glass aquaria (610 mm x 305 mm x 310 mm), with a fill volume of 45 L. Progeny exposure tanks were 12 L glass aquaria (305 x 205 x 210 mm), with a fill volume of 9.5 L. It was not specified if larval chambers had drains to allow for water level reduction.

Guideline Criteria	Reported Information
Embryo and Fry Chambers 120 mL glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen. Chambers can be oscillated vertically using rocker arm apparatus (2 rpm motor) or placed in separate chambers with self-starting siphons.	157 mL glass tubing (50-mm diameter, 80 mm length) and 0.47 mm^2 nylon mesh screen bottom. The embryo cups were suspended in the water column of each chamber and oscillated vertically (2 rpm).
Flow Rate Flow rates to adult tanks or larval chambers should provide 90% replacement in 8-12 hours, and maintain DO at above 75% of saturation. The toxicant level cannot drop below 20% with fish in the tank.	The flow-splitting devices supplied approximately 7 tank volumes per day to each of the adult tanks and approximately 7.5 tank volumes per day to each of the progeny tanks. The DO ranged from 4.6 to 8.4 mg/L during the study (56-102% saturation).
Aeration Dilution water should be aerated to insure dissolved oxygen concentrations at or near 100% saturation. Test tanks and embryo chambers should not be aerated.	Test solutions were not aerated during the study.

C. Chemical System

Guideline Criteria	Reported Information
Nominal Concentrations Minimum of 5 concentrations and a control, all replicated; plus solvent control if appropriate. Toxicant conc. must be measured in one tank at each toxicant level every week.	0 (negative and solvent controls), 0.09, 0.22, 0.52, 1.25, and 3.00 μg/L. Toxicant concentrations were measured from one tank in each treatment group at least once per week.

Guideline Criteria	Reported Information
Other Variables 1. DO must be measured at each conc. at least once a week.	1. DO was measured in each replicate aquaria at test initiation and at least once weekly during the test.
2. Test water temp. must be recorded continuously.	2. Temperature measured in each replicate aquaria at test initiation and twice weekly during the test. Temperature was also continuously monitored in two solvent control and 1.25 μg/L replicates.
3. Freshwater: A control and one conc. must be analyzed weekly for pH, alkalinity, hardness, and conductance. Natural seawater: must maintain a constant salinity and not fluctuate more than 6% weekly; monthly pH range <0.8 pH units.	3. pH was measured in each replicate aquaria at test initiation and at least once weekly during the test. Conductivity, alkalinity, and total hardness were determined once per week in two solvent control and 1.25 μg/L replicates.
Solvents Should not exceed 0.1 ml/L in a flow-through system. Acceptable solvents are: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.	Dimethylformamide (DMF), 2.86 μL/L

Comments: None.

12. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. This study was conducted in compliance with GLP standards set forth by the United Kingdom, OECD and U.S. EPA.
Data Endpoints must include: · survival of F_0 and F_1 embryos, time required to hatch, and hatching success; · survival and total length of F_0 fish at 4 and 8 weeks after hatching; · weights and lengths of F_1 fish at 8 weeks; · incidence of pathological or histological effects; and · observations of other effects or clinical signs.	Data Endpoints included: · survival of F_0 and F_1 embryos and hatching success; · survival of F_0 fish at 8 and 21 weeks after hatching; total length and wet weights of F_0 fish at 21 and 44 weeks after hatching · total lengths and wet weights of surviving F_1 fish at 8 weeks · other effects and clinical signs of toxicity
Raw data included?	Not sufficient. Replicate data were not provided for survival (F_0 and F_1 generations) or growth at Weeks 21 and 44 (F_0 generation). Therefore, statistical verification for these endpoints was not conducted.

F_0 Results:

Nominal Conc. (µg/L)	Mean Measured Conc. (µg a.i./L)	% Hatch	8 Week Post-Hatch % Survival	21 Week Post-Hatch % Survival	Test Termination (Day 315) % Survival
Negative Control	<0.020	99.5	89	94	NR
Solvent Control	<0.019	99.0	90	94	NR
0.09	0.061	99.0	95	96	NR
0.22	0.20	95.6	90	96	NR
0.52	0.44	97.5	86	94	NR
1.25	1.1	97.0	92	96	NR
3.00	2.4	97.5	94	98	NR

NR= Not reported

Mean Measured Conc (µg a.i./L)	Mean Total Length (mm)				Wet Weight (g)			
	Week 21		Week 44		Week 21		Week 44	
	♂	♀	♂	♀	♂	♀	♂	♀
<0.020	64.4	49.4	79.1	63.7	5.30	2.10	10.0	4.79
<0.019	61.7	47.7	80.0	59.4	4.90	1.96	10.2	4.35
0.061	65.6	48.8	80.5	59.8	5.53	2.11	10.6	3.94
0.20	66.5*	50.0	79.3	60.9	5.61	2.17	10.1	4.48
0.44	67.2*	49.7	80.7	62.1	5.68	2.27	11.3	5.12
1.1	67.1*	46.0	80.9	59.1	6.12*	1.92	11.8*	4.06
2.4	66.8*	49.0	82.0*	61.6	6.05*	2.20	10.9	4.45

* Statistically different from solvent control and pooled controls.

Mean Measured Conc. (μg a.i./L)	Number of Spawns	Total Number of Eggs	Number of Eggs/ Spawn	Number of Spawns/ Female	Number of Eggs/ Female	Number of Eggs/ Female/ Day
<0.020	NR	NR	NR	NR	2382	15.0
<0.019	NR	NR	NR	NR	2314	14.2
0.061	NR	NR	NR	NR	2175	12.8
0.20	NR	NR	NR	NR	2886	17.7
0.44	NR	NR	NR	NR	1644	10.8
1.1	NR	NR	NR	NR	1992	11.7
2.4	NR	NR	NR	NR	3104	18.9

NR= Not reported

Toxicity Observations: No treatment-related effects were observed on hatching success of the F_0 generation (Table 12, p. 41), and no treatment-related effects were observed on survival of the F_0 generation after 8 or 21 Weeks post-hatch (Table 2, p. 31). F_0 survival was not determined at test termination.

Compared to the solvent control and pooled controls, statistically-significant increases in male lengths and/or wet weights and Weeks 21 and 44 were observed at the $\geq$0.20 μg/L levels. However, since corresponding female fish of the same age were not affected, these results were regarded as not biologically significant and were not used in the NOEC and LOEC estimations (Tables 4-7, pp. 33-36).

No significant treatment-related effects were observed for egg production (number of eggs per female and number of eggs per female per reproductive day; Tables 13-14, pp. 42-43). The treatment groups were compared to the solvent control and pooled controls.

F_1 Results:

Mean Measured Concentration (μg a.i./L)	% Hatch	8 Week Post-Hatch % Survival		8 Week Post-Hatch Length (mm)		8 Week Post-Hatch Wet Weight (g)	
		ELS1	ELS2	ELS1	ELS2	ELS1	ELS2
<0.020	73.6	97	96	33.9	32.0	0.68	0.58
<0.019	83.3	92	92	31.8	33.7	0.59	0.67
0.061	88.6	95	87	33.0*	34.7*	0.60	0.73
0.20	90.3	99	99	32.4	33.8	0.59	0.67
0.44	66.3	82*	78*	32.0*	33.4	0.60	0.69
1.1	87.0	91	95	33.0*	33.3	0.64	0.70
2.4	85.8	97	93	32.2	34.1	0.54*	0.71

* Statistically different from solvent control and/or pooled controls.

Toxicity Observations: No treatment-related effects were observed on hatching success of the F_1 generation when compared to pooled controls (Table 15, p. 44). At 8 Weeks post-hatch, a statistically-significant reduction in survival was observed at the 0.44 μg/L level during both ELS studies (Table 3, p. 32). However, since no dose-dependent response was observed at higher levels, the differences were regarded as not biologically significant and were not used in the NOEC and LOEC estimations.

At 8 Weeks post-hatch, statistically-significant decreases in length were observed between the solvent control group and the 0.061 (both ELS studies), 0.44, and 1.1 μg/L test concentrations (Tables 8 and 10, pp. 37 and 39). The magnitudes of these differences were very small (0.6-3.8%), no significant decreases in weight were observed at the corresponding concentrations, and there was no relationship between exposure concentration and biological response. Therefore, these results were regarded as not biologically significant and were not used in the NOEC and LOEC estimations.

At 8 Weeks post-hatch, a statistically significant reduction in wet weight was observed at the 2.4 μg/L treatment group of the first ELS test, compared to the solvent control (Tables 9 and 11, pp. 38 and 40). The magnitude of this reduction was small (8.5% compared to the solvent control) and the other ELS study recorded a small (6.0%) but not significant increase in weight compared with the solvent control. Consequently, this difference was regarded as not biologically significant and was not used in the NOEC and LOEC estimations.

B. Reported Statistical Results

Data obtained for the F_0 generation that were statistically analyzed included (1) hatching success, (2) survival at 8 and 21 Weeks post-hatch, (3) total length and wet weight at 21 and 44 Weeks post-hatch (gender-specific), (4) total number of eggs produced, and (5) the total number of eggs produced per female reproductive day. Data obtained for the F_1 generation that were statistically analyzed included (1) hatching success, (2) survival at 8 Weeks post-hatch, (3) and total length and wet weight at 8 Weeks post-hatch.

Survival data were analyzed using contingency table tests to identify treatment groups that showed a statistically-significant difference ($p \leq 0.05$) from the solvent control or pooled control groups. The growth (length and weight) data and reproduction (egg hatchability and production) data were evaluated for normality and for homogeneity of variance prior to analysis of variance (ANOVA) or non-parametric techniques. Comparisons were then made between the test groups and solvent control or pooled control data using Dunnett's t-test (one- or two-sided) or a non-parametric procedure. If assumptions for ANOVA were not met, data were transformed (square root or log), and if the data still failed to meet the assumptions for analysis, Wilcoxon's Rank Sum Test or Steel's Many One-Rank Test was used to identify significant differences.

The no observed effect concentration (NOEC) is the highest tested concentration at which a measured biological parameter is not statistically different (at the 95% confidence interval) than the control. The lowest observed effect concentration (LOEC) is the lowest tested concentration at which any measured biological parameter is statistically different from the control and above which all concentrations are significantly different. The results are based on nominal test concentrations.

Biological Endpoint	NOEC (μg/L)	LOEC (μg/L)
F_0 hatching success	3.0	>3.0
F_0 8-Week post-hatch survival	3.0	>3.0
F_0 21-Week post-hatch survival	3.0	>3.0
F_0 21-Week post-hatch length (male and female)	3.0	>3.0
F_0 21-Week post-hatch weight (male and female)	3.0	>3.0
F_0 44-Week post-hatch length (male and female)	3.0	>3.0
F_0 44-Week post-hatch weight (male and female)	3.0	>3.0
F_0 # of spawns/female	NA	NA
F_0 # of eggs/female/reproductive day	3.0	>3.0
F_1 hatching success	3.0	>3.0
F_1 8-Week post-hatch survival (ELS1)	3.0	>3.0
F_1 8-Week post-hatch length (ELS1)	3.0	>3.0
F_1 8-Week post-hatch weight (ELS1)	3.0	>3.0
F_1 8-Week post-hatch survival (ELS2)	3.0	>3.0
F_1 8-Week post-hatch length (ELS2)	3.0	>3.0
F_1 8-Week post-hatch weight (ELS2)	3.0	>3.0

NA - Not assessed

NOEC: 3.0 μg/L **LOEC:** >3.0 μg/L **MATC:** Not determined

13. REVIEWER'S STATISTICAL RESULTS:

No endpoints assessed were significantly affected by treatment with Novaluron technical. Data obtained for the F_0 generation that were statistically analyzed included hatching success,

the total number of eggs produced, and the total number of eggs produced per female reproductive day. Data obtained for the F_1 generation that were statistically analyzed included hatching success, and total length and wet weight at 8 Weeks post-hatch. For all endpoints, a t-test was used to compare response between the solvent and negative controls and no differences were found. As a result, treatment groups were compared to the pooled controls for all analyses. All data were analyzed to determine if they met the assumptions of normal distribution and homogeneous variances. If they met these assumptions, data were analyzed using ANOVA followed by Bonferroni's and William's tests. If they did not meet these assumptions, data were analyzed using the non-parametric Kruskal-Wallis test. All analyses were conducted using TOXSTAT statistical software and mean-measured concentrations.

Replicate data were not provided for survival data (F_0 generation at 8 and 21 Weeks post-hatch and F_1 generation at 8 Weeks post-hatch), or for F_0-generation growth data at 21 and 44 Weeks post-hatch. Therefore, these endpoints were not statistically verified.

14. REVIEWER'S COMMENTS:

This study is classified INVALID, since adequate raw data pertaining to survival of both generations and growth of the F_0 generation were not provided to thoroughly verify the results of the study. In addition, guideline requirements for a life-cycle toxicity study were not met, since several significant endpoints were not measured, i.e., the time to hatch for both F_0 and F_1 generations, lengths of F_0 fish at 4 and 8 Weeks post-hatch, and survival of F_0 fish at 4 Weeks post-hatch. This study may be upgraded to Supplemental status if appropriate raw data are provided.

Novaluron residues in the control and treatment group fish were determined from sample fish shipped to Huntingdon life Sciences (Makhteshim project number R-14842). The mean residues were determined in the carcass, muscle, and viscera of the fish.

Analysis of the test waters on Day 292 revealed that the concentration in the 0.22 μg/L tank was approximately twice the target (footnote, p. 30). Analysis of the syringe concentrates revealed that on Day 286, the 0.22 and 0.52 μg/L syringes had inadvertently been filled with the opposing concentrate, hence increasing the 0.22 μg/L target test concentration and reducing the 0.52 μg/L target test concentration for a period of 7 days. On Day 294, the syringes were re-filled with the correct concentrates.

It was not specified if larval chambers had drains to allow for water level reduction.

5. REFERENCES:

Comb, A.L. 1997. Rimon (Pure): Determination of the physico-chemical properties.

Brownlee, K.A. 1965. Fisher's Exact Test For 2 X 2 Tables. Statistical Theory and Methodology in Science and Engineering. 2nd Edition. John Wiley and Sons, 163-166.

D'Agostine, R.B. 1971. An omnibus test of normality for moderate and large size samples. Biometrika 58, 342-348.

Shapiro, S.S. and M.B. Wilk. 1965. An analysis of variance test for normality (complete samples). Biometrika 52, 591-611.

Snedecor, G.W. and W.G. Cochran. 1980. Statistical Methods, 252-253.

Hollander, M. and D.A. Wolfe. 1973. Non-parametric Statistical Methods. J. Wiley & Sons Inc.

Steel, R.G. 1959. A multiple comparison rank sum test: treatments versus control. Biometrics, 15, 560-572.

16. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Length F1E1 56 dph
File: 58051156 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	2.998	0.600	0.258
Within (Error)	8	18.623	2.328	
Total	13	21.620		

Critical F value = 3.69 (0.05,5,8)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

Length F1E1 56 dph
File: 58051156 Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	32.875	32.875		
2	0.061	33.000	33.000	-0.095	
3	0.20	32.650	32.650	0.170	
4	0.44	31.650	31.650	0.927	
5	1.1	32.950	32.950	-0.057	
6	2.4	32.200	32.200	0.511	

Bonferroni T table value = 2.90 (1 Tailed Value, P=0.05, df=8,5)

Length F1E1 56 dph
File: 58051156 Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	4			
2	0.061	2	3.828	11.6	-0.125
3	0.20	2	3.828	11.6	0.225
4	0.44	2	3.828	11.6	1.225
5	1.1	2	3.828	11.6	-0.075
6	2.4	2	3.828	11.6	0.675

Length F1E1 56 dph
File: 58051156 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	32.875	32.875	32.917
2	0.061	2	33.000	33.000	32.917
3	0.20	2	32.650	32.650	32.650
4	0.44	2	31.650	31.650	32.300
5	1.1	2	32.950	32.950	32.300
6	2.4	2	32.200	32.200	32.200

Length F1E1 56 dph
File: 58051156 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	32.917				
0.061	32.917	0.032		1.86	k= 1, v= 8
0.20	32.650	0.170		1.96	k= 2, v= 8
0.44	32.300	0.435		2.00	k= 3, v= 8
1.1	32.300	0.435		2.01	k= 4, v= 8
2.4	32.200	0.511		2.02	k= 5, v= 8

s = 1.526
Note: df used for table values are approximate when v > 20.

weight F1E1 56 dph
File: 5805w156 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.017	0.003	0.500
Within (Error)	8	0.049	0.006	
Total	13	0.066		

Critical F value = 3.69 (0.05,5,8)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

weight F1E1 56 dph
File: 5805w156 Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	0.640	0.640		
2	0.061	0.600	0.600	0.596	
3	0.20	0.595	0.595	0.671	
4	0.44	0.595	0.595	0.671	
5	1.1	0.635	0.635	0.075	
6	2.4	0.535	0.535	1.565	

Bonferroni T table value = 2.90 (1 Tailed Value, P=0.05, df=8,5)

weight F1E1 56 dph
File: 5805w156 Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	4			
2	0.061	2	0.194	30.4	0.040
3	0.20	2	0.194	30.4	0.045
4	0.44	2	0.194	30.4	0.045
5	1.1	2	0.194	30.4	0.005
6	2.4	2	0.194	30.4	0.105

weight F1E1 56 dph
File: 5805w156 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	0.640	0.640	0.640
2	0.061	2	0.600	0.600	0.606
3	0.20	2	0.595	0.595	0.606
4	0.44	2	0.595	0.595	0.606
5	1.1	2	0.635	0.635	0.606
6	2.4	2	0.535	0.535	0.535

weight F1E1 56 dph
File: 5805w156 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM

GRPS 1&2 POOLED	0.640				
0.061	0.606	0.499		1.86	k= 1, v= 8
0.20	0.606	0.499		1.96	k= 2, v= 8
0.44	0.606	0.499		2.00	k= 3, v= 8
1.1	0.606	0.499		2.01	k= 4, v= 8
2.4	0.535	1.552		2.02	k= 5, v= 8

s = 0.078
Note: df used for table values are approximate when v > 20.

length F1E2 56 dph
File: 58051256 Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	GRPS 1&2 POOLED	32.950	32.950	21.000
2	0.061	34.700	34.700	25.000
3	0.20	33.850	33.850	17.000
4	0.44	33.050	33.050	12.500
5	1.1	33.400	33.400	11.000
6	2.4	34.150	34.150	18.500

Calculated H Value = 5.137 Critical H Value Table = 11.070
Since Calc H < Crit H **FAIL TO REJECT Ho:All groups are equal.**

length F1E2 56 dph
File: 58051256 Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 1 4 5 3 6 2
1	GRPS 1&2 POOLED	32.950	32.950	\
4	0.44	33.050	33.050	. \
5	1.1	33.400	33.400	. . \
3	0.20	33.850	33.850	. . . \
6	2.4	34.150	34.150	. . . . \
2	0.061	34.700	34.700	. . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps - multiple SE values

weight F1E2 56 dph
File: 5805w256 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.021	0.004	1.333
Within (Error)	8	0.022	0.003	
Total	13	0.043		

Critical F value = 3.69 (0.05,5,8)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

weight F1E2 56 dph
File: 5805w256 Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	0.628	0.628		
2	0.061	0.735	0.735	-2.266	
3	0.20	0.670	0.670	-0.896	
4	0.44	0.690	0.690	-1.318	
5	1.1	0.705	0.705	-1.634	
6	2.4	0.715	0.715	-1.845	

Bonferroni T table value = 2.90 (1 Tailed Value, P=0.05, df=8,5)

weight F1E2 56 dph
File: 5805w256 Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	4			
2	0.061	2	0.137	21.9	-0.108
3	0.20	2	0.137	21.9	-0.042
4	0.44	2	0.137	21.9	-0.062
5	1.1	2	0.137	21.9	-0.077
6	2.4	2	0.137	21.9	-0.087

weight F1E2 56 dph
File: 5805w256 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	4	0.628	0.628	0.628
2	0.061	2	0.735	0.735	0.698
3	0.20	2	0.670	0.670	0.698
4	0.44	2	0.690	0.690	0.698
5	1.1	2	0.705	0.705	0.705
6	2.4	2	0.715	0.715	0.715

weight F1E2 56 dph
File: 5805w256 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	0.628				
0.061	0.698	1.565		1.86	k= 1, v= 8
0.20	0.698	1.565		1.96	k= 2, v= 8
0.44	0.698	1.565		2.00	k= 3, v= 8
1.1	0.705	1.713		2.01	k= 4, v= 8
2.4	0.715	1.934		2.02	k= 5, v= 8

s = 0.052
Note: df used for table values are approximate when v > 20.

% hatch F0
File: 5805h Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	GRPS 1&2 POOLED	99.250	99.250	525.000
2	0.061	99.000	99.000	250.000
3	0.20	95.625	95.625	229.000
4	0.44	97.500	97.500	210.000
5	1.1	97.000	97.000	167.500
6	2.4	97.500	97.500	214.500

Calculated H Value = 5.755 Critical H Value Table = 11.070
Since Calc H < Crit H **FAIL TO REJECT Ho:All groups are equal.**

% hatch F0
File: 5805h Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 3 5 4 6 2 1
3	0.20	95.625	95.625	\
5	1.1	97.000	97.000	. \
4	0.44	97.500	97.500	. . \
6	2.4	97.500	97.500	. . . \
2	0.061	99.000	99.000	. . . . \
1	GRPS 1&2 POOLED	99.250	99.250	. . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps - multiple SE values

egg production
File: 5805ep Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	11760564.444	2352112.889	0.540
Within (Error)	46	200519909.249	4359128.462	
Total	51	212280473.692		

Critical F value = 2.45 (0.05,5,40)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

egg production
File: 5805ep Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	2345.933	2345.933		
2	0.061	2174.833	2174.833	0.170	
3	0.20	2886.143	2886.143	-0.565	
4	0.44	1643.875	1643.875	0.768	
5	1.1	1991.875	1991.875	0.387	
6	2.4	3104.125	3104.125	-0.829	

Bonferroni T table value = 2.42 (1 Tailed Value, P=0.05, df=40,5)

egg production
File: 5805ep Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	15			
2	0.061	6	2444.673	104.2	171.100
3	0.20	7	2316.590	98.7	-540.210
4	0.44	8	2215.673	94.4	702.058
5	1.1	8	2215.673	94.4	354.058
6	2.4	8	2215.673	94.4	-758.192

egg production
File: 5805ep Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	15	2345.933	2345.933	2216.523
2	0.061	6	2174.833	2174.833	2216.523
3	0.20	7	2886.143	2886.143	2216.523
4	0.44	8	1643.875	1643.875	2216.523
5	1.1	8	1991.875	1991.875	2216.523
6	2.4	8	3104.125	3104.125	3104.125

egg production
File: 5805ep Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	2216.523				
0.061	2216.523	0.128		1.68	k= 1, v=46
0.20	2216.523	0.135		1.76	k= 2, v=46
0.44	2216.523	0.142		1.79	k= 3, v=46
1.1	2216.523	0.142		1.80	k= 4, v=46
2.4	3104.125	0.829		1.80	k= 5, v=46

s = 2087.853
Note: df used for table values are approximate when v > 20.

eggs per female per reproductive day
File: 5805epr Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	419.604	83.921	0.591
Within (Error)	46	6527.514	141.902	
Total	51	6947.118		

Critical F value = 2.45 (0.05,5,40)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

eggs per female per reproductive day
File: 5805epr Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	14.553	14.553		
2	0.061	12.800	12.800	0.305	
3	0.20	17.674	17.674	-0.572	
4	0.44	10.775	10.775	0.724	
5	1.1	11.725	11.725	0.542	
6	2.4	18.963	18.963	-0.845	

Bonferroni T table value = 2.42 (1 Tailed Value, P=0.05, df=40,5)

eggs per female per reproductive day
File: 5805epr Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	15			
2	0.061	6	13.948	95.8	1.753
3	0.20	7	13.217	90.8	-3.121
4	0.44	8	12.642	86.9	3.778
5	1.1	8	12.642	86.9	2.828
6	2.4	8	12.642	86.9	-4.409

eggs per female per reproductive day
File: 5805epr Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	15	14.553	14.553	13.610
2	0.061	6	12.800	12.800	13.610
3	0.20	7	17.674	17.674	13.610
4	0.44	8	10.775	10.775	13.610
5	1.1	8	11.725	11.725	13.610
6	2.4	8	18.963	18.963	18.963

eggs per female per reproductive day
File: 5805epr Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	13.610				
0.061	13.610	0.164		1.68	k= 1, v=46
0.20	13.610	0.173		1.76	k= 2, v=46
0.44	13.610	0.181		1.79	k= 3, v=46
1.1	13.610	0.181		1.80	k= 4, v=46
2.4	18.963	0.845		1.80	k= 5, v=46

s = 11.912
Note: df used for table values are approximate when v > 20.

average hatchability F1
File: 5805hf1 Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	GRPS 1&2 POOLED	79.380	79.380	291.000
2	0.061	89.850	89.850	191.000
3	0.2	90.100	90.100	152.000
4	0.44	71.029	71.029	124.500
5	1.1	86.663	86.663	211.000
6	2.4	88.688	88.688	255.500

Calculated H Value = 8.106 Critical H Value Table = 11.070
Since Calc H < Crit H **FAIL TO REJECT Ho:All groups are equal.**

average hatchability F1
File: 5805hf1 Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 4	0 1	0 5	0 6	0 2	0 3
4	0.44	71.029	71.029	\					
1	GRPS 1&2 POOLED	79.380	79.380	.	\				
5	1.1	86.663	86.663	.	.	\			
6	2.4	88.688	88.688	.	.	.	\		
2	0.061	89.850	89.850	.	.	.	.	\	
3	0.2	90.100	90.100	.	.	.	.	.	\

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps - multiple SE values